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EFFECTS OF JUVENILE HORMONE ANALOGUES ON THE METAMORPHOSIS OF BEETLES *TROGODERMA GRANARIUM* (DERMESTIDAE) AND *CARYEDON GONAGRA* (BRUCHIDAE)

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Several authors—for example Bowers and Thompson (1963) in a paper on *Tenebrio molitor* (Tenebrionidae), Sláma and associates in a study on *Dermestes vulpinus* (Dermestidae), and De Wilde (1971) in a note concerning *Leptinotarsa decemlineata* (Chrysomellidae)—demonstrated that application of juvenile hormone analogues (JHa) to freshly molted pupae of beetles inhibits imaginal differentiation. Detailed description of the effects elicited, however, is available only for the flour beetle, *Tenebrio molitor* (Rose, Westermann, Trautmann, Schmralek and Klauske 1969). This species is also the only beetle in which the effects of JHa on the larvae were studied (Schmialek, 1963). Therefore, in the present study we have examined in detail the effects of JHa on both the larval-pupal and pupal-adult transformation of representatives of the coleopteran families Dermestidae and Bruchidae.

The dermestids and bruchids differ from one another in many features, and it was intriguing to compare their responses to JHa. Both families include serious pests. The khapra beetle, *Trogoderma granarium*, is polyphagous and infests stored grains, malt, seeds, flower, dry milk products, and woolen cloths in many parts of the world, particularly in hot dry regions (Hadaway, 1956). The hairy larvae undergo at least 4 larval molts in males and 5 in females but the number of larval instars may more than double. The larvae may diapause for more than a year at a temperature around 20° C. The last larval instar of nondiapausing insects lasts 13 days (including 2 days of the prepupal stage) and the pupal instar lasts 7–8 days. The pupae, which are also covered with hairs, remain in the last larval exuvia until completion of adult development.

By contrast, the groundnut beetle, *Caryedon gonagra*, attacks only, or at least primarily, the stores of peanuts in West Africa (Davey, 1959). After hatching, its grub-like larvae bore into the seeds and remain inside until the fourth larval instar. The larvae leave seeds 13–14 days after ecdysis into the fourth instar to spin cocoons. The spinning continues for about 1 day; then the larvae rest inside the cocoons for 2–3 days before ecdysing as pupae. The pupal instar lasts 7–8 days. The adults escape from the cocoons a few days after imaginal ecdysis.

Material and Methods

(1.) *The juvenile hormone analogues*

Twenty-seven analogues were selected to include compounds with diverse chemical structures (Fig. 1). The substances were prepared and kindly provided

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by Drs. M. Romaňuk (Nos. IV, IX, X, XI, XVI, XVII, XVIII); Václav Jarolím (Nos. III, VII, VIII, XIII, XIV, XV); K. Hejno (Nos. I, II); Z. Arnold (Nos. XX, XXI, XXII, XXIII); P. Beran (Nos. XXV, XXVI); and J. Kahovcová (No. XXVII)—all of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences—and by Dr. J. B. Siddall of the Zoecon Corporation, Palo Alto, California (Nos. V, VI, XII, XIX, XXIV). In most experiments the compounds were dissolved in acetone (grade *pro chromatography*, product of Lachema, Brno) in the ratio 1 : 10, 1 : 100, 1 : 1000 etc.; the solutions were stored at —10° C for up to six months.

(2.) *Biological tests*

Both species of beetles were reared on roasted peanuts in constant darkness at 27° C and 60–70% R.H. The last instar larvae or freshly ecdysed pupae were collected daily from the stock culture and kept in groups of 10 specimens in Petri dishes under the same regime. Fresh food was provided every second week. The insects selected for the expriments (females were used in most cases) were treated with 0.5 μl (*Caryedon*) or 1 μl (*Trogoderma*) of a solution of JHa on the dorsal body side. No anesthesia was used. The solutions were dispensed from a microsyringe driven by means of a screw. In the case of prepupae and pupae of *Caryedon*, the syringe-needle was inserted through the cocoon. Since the pupae of both species were sensitive to oily materials (10% medicinal olive oil in acetone killed 50% of pupae) the highest concentration of analogues used in most experiments was 1%. Application of 1% olive oil in acetone to the controls had no effect.

In a few experiments, the compounds were administered in the form of vapors. To this end a measured amount of analogue was dissolved in 0.5 ml acetone and soaked into a disk of filter paper (8 cm in diameter); the solvent was evaporated and the impregnated paper put in the bottom of a glass Petri dish (diameter 9 cm). Larvae or pupae of *Trogoderma* were placed in an uncovered dish (diameter 4 cm) and the latter was put upon the filter paper. The Petri dish was covered with a lid and the entire assembly was placed in another covered Petri dish (diameter 14 cm).

The treated larvae were periodically observed until they either died or developed into adults. The number of ecdyses was recorded and all ecdysed insects were examined for morphological abnormalities; it was often necessary to remove old exuvia with forceps. Effects on pupae were evaluated two days after the controls had emerged as adults; ordinarily the affected insects produced a new cuticle but could not escape from the old exuvia. The specimens that died prior to the deposition of a new cuticle were disregarded in evaluating results.

Results

(1.) *Action of the juvenile hormone analogues on pupae*

Pupae treated with JHa often molted into various intermediate forms (Fig. 2) that were classified with the aid of a scoring system (Table I) based on the ratio of pupal and adult characters. The maximum effect, *i.e.*, formation of a perfect second pupa, was never observed in either species. Even the most affected specimens of *Trogoderma* displayed slightly pigmented adult eyes, outlines of the segmentation of appendages, and, most important, lacked the pupal hairs. The maximally affected individuals of *Caryedon* resembled normal pupae but their eyes

and appendages also showed adult differentiation. In the less influenced insects of either species, the adult characteristics gradually spread from the head and thorax while the pupal features simultaneously disappeared; the distribution of

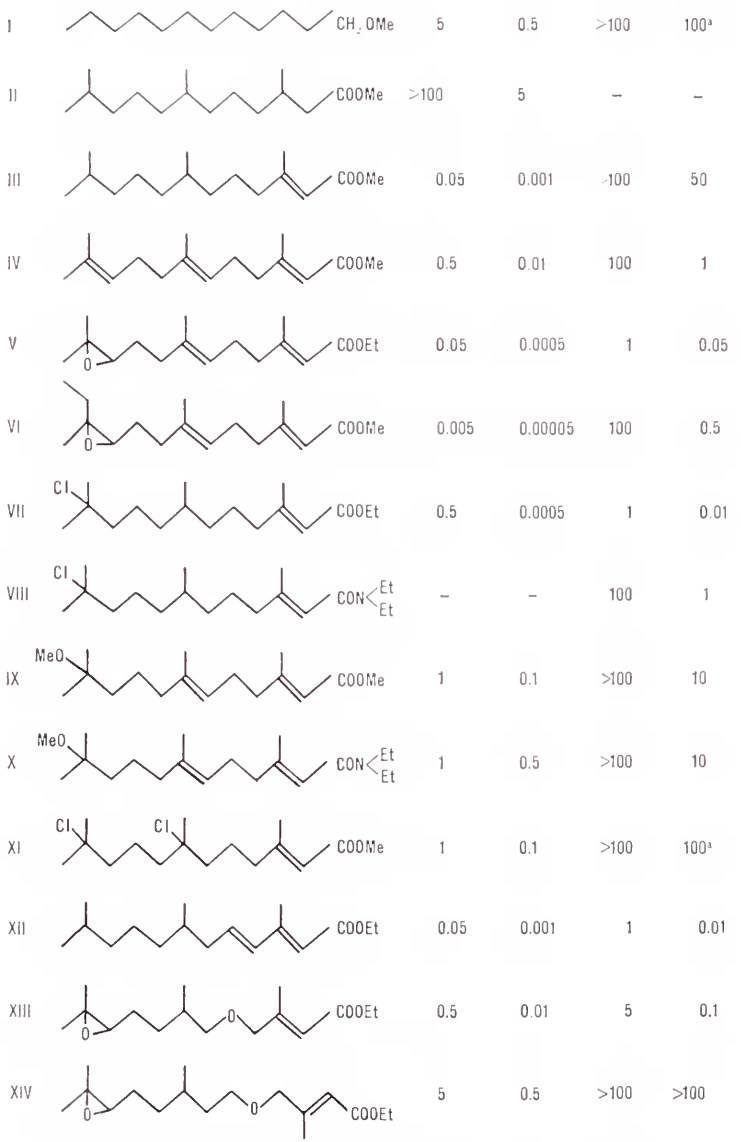
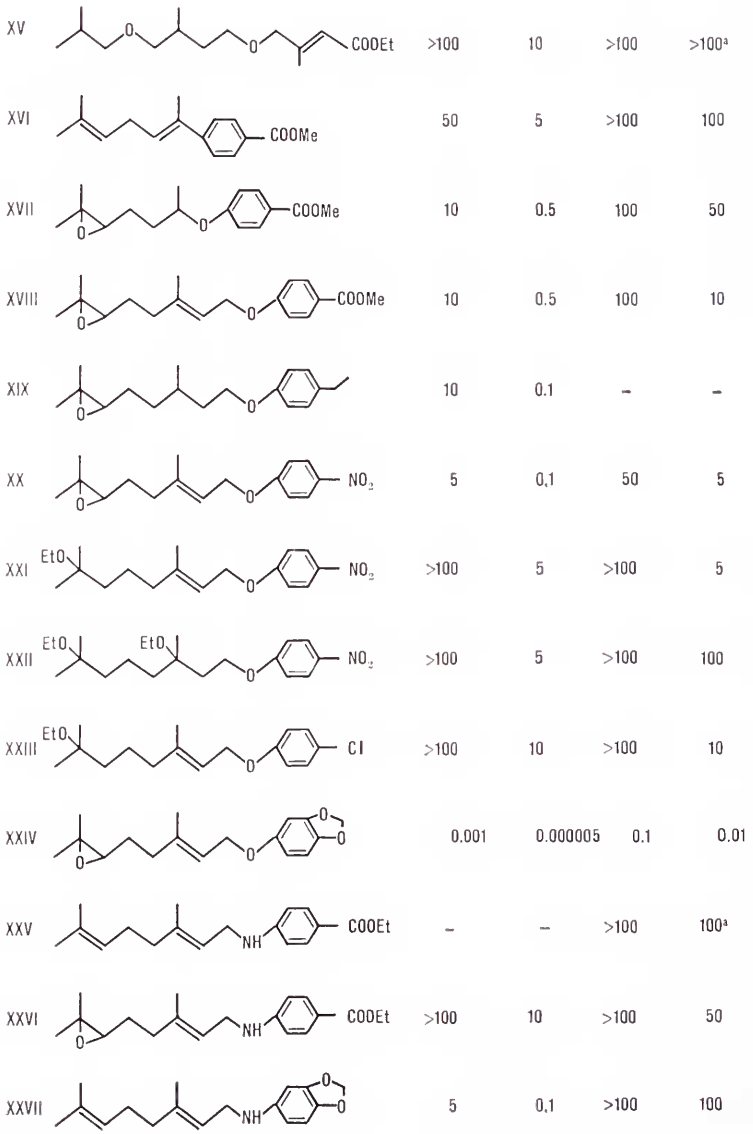


FIGURE 1. Activities of the juvenile hormone analogues in pupal assays. The compounds (all racemic mixture except VI which was *trans, trans, cis* isomer; the aliphatic substances contained mostly two thirds of the 2-*trans* isomers) were applied in 0.5 μl (*Caryedon*) and 1 μl (*Trogoderma*) of acetone on the surface of freshly ecdysed pupae. The elicited effects were scored as described in Table I and are expressed here in ID_{50} and LD_{50}—amount of the com-

pupal and adult characteristics always followed a precise spatial pattern. The least affected animals appeared as normal adults except that their wings were crumpled and, in the case of *Trogoderma*, the cuticle on the dorsal side of abdomen



pound (in μg) provoking in the average the effect of score 2; LD_{50}—amount of the compound (in μg) causing small [scores (1) and 1] but nevertheless lethal effects in 50% of treated insects. The doses marked (a) were toxic. A pupa of *Trogoderma* weighed about 3.5 mg and that of *Caryedon* about 18 mg. Ten pupae, 0–24 hrs after ecdysis, were used in each assay.

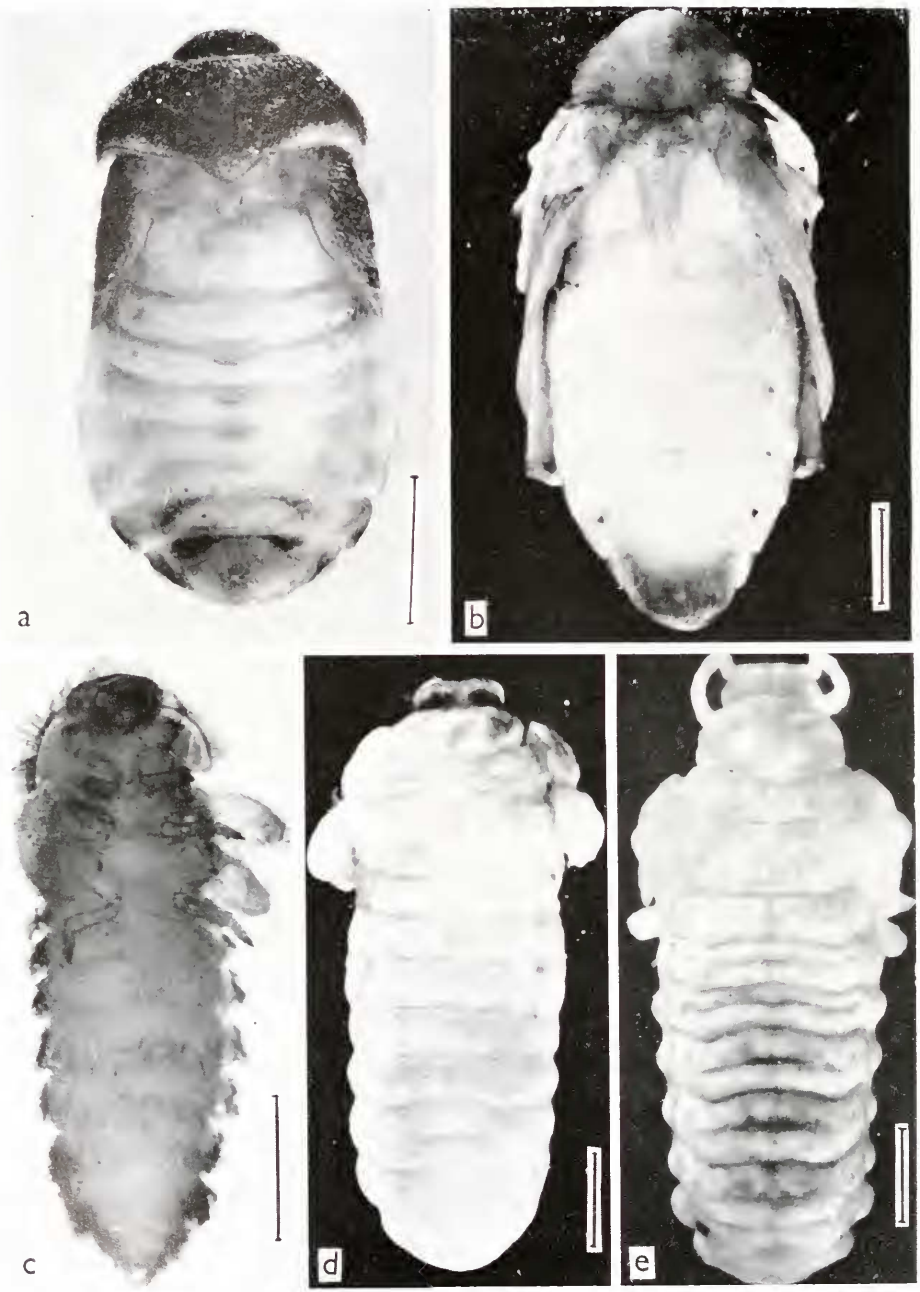


FIGURE 2. The pupal-adult (top) and larval-pupal (bottom) intermediates produced by JHa in *Trogoderma* (pictures a and c) and *Caryedon* (pictures b, d, and e). The pupal-adult intermediates were classified with score 2, the larval-pupal ones with score 4 (pictures c and d) and 2 (picture e). The lines indicate length of 1 mm.

was not completely pigmented. These insects were probably affected internally because they behaved like insects with external juvenile hormone effects and died without escaping from the old exuvia.

Some of the pupal-adult intermediates showed a deep depression in the integument on either side of the prothorax. These depressions were probably caused by contractions of muscles attached to the corresponding parts of the integument at the time when the newly formed cuticle had not yet hardened. Treatments with high concentrations of the analogues occasionally inhibited sclerotization of the emerging insects. This effect was also observed after treatments with olive oil and its cause was obscure. A rare effect of JHa was prolongation of the pupal instar; in this case the treated insects ecdysed as adults up to three times later than the controls.

TABLE I

Scoring system for assessing juvenile effects in pupal assays

Score	Characteristic features	
	Trogoderma granarium	*Caryedon gonagra*
0	Normal adult	Normal adult.
(1)	Adult lacking the cuticular tanning in small areas of abdominal tergites or having crumpled wings.	Adult with crumpled wings or adult failing to extract some body parts from the pupal exuvia.
1	Virtually normal adult remaining in the pupal exuvia. Wings point downwards as in pupa.	Virtually normal adult remaining in the pupal exuvia; wings point downwards as in pupa.
2	Head and thorax are nearly of adult form but large portions of abdominal tergites remain untanned.	The cuticular tanning is lighter than in adults and the number of adult hairs is reduced; wings often appear swollen.
3	Head and thorax are predominantly of adult form but most of the abdomen is covered with a pupal-like cuticle lacking pupal hairs.	Head and thorax are of nearly adult form but the eyes are more remote from one another than in the adults and their pigment is incompletely developed; the abdomen remains white as in pupae; wings are often swollen.
4	Head and thorax are intermediate between pupa and adult; appendages are virtually adult; wings are slightly tanned; the abdomen is pupal-like except for the lack of pupal hairs.	Nearly perfect second pupa but appendages are differentiated and the tips of mandibles sclerotized; wings often stretch at right angles from the body.
5	Perfect second pupa.	Perfect second pupa.

The morphological effects were most pronounced when the compounds were applied immediately after the pupal ecdysis. Pupae 0–24 hrs old were therefore used for assaying activities of different JHa. Since the effects produced by some treatments varied as much as over 3 grades of our scoring system (10 insects were used in each assay), the average effect was calculated for each concentration tested. The activity of each analogue was expressed in terms of the dose provoking an average effect of grade 2 (ID_{50}; *cf.* Sláma,, 1971) and also in terms of the critical dose causing small but lethal developmental derangements [grades 1 and (1)] in 50% of treated insects (LD_{50}).

Figure 1, which summarizes the results, shows that the compounds differ considerably in their activities. In the case of *Trogoderma* pupae, LD_{50} of the best compounds ranged from several picograms to a few nanograms; in the case of *Caryedon* pupae ten or more nanograms were required. With each of the most active materials the range of effects from score 1 to score 4 was realized within 10^4–10^7 increase in dose. Less active compounds produced only small effects even when applied in the highest amounts and a few others had no effect on the development of *Caryedon*.

(2.) *Action of JHa on* Trogoderma *larvae*

Larvae treated within the first nine days of the last instar (the total length of the instar was 13 days) underwent up to six extra larval molts (Table II) and

TABLE II

Development of Trogoderma *larvae treated with 3,4-methylendioxyphenyl 6,7-epoxygeranyl ether (compound No. XXIV)*+

Dose (μg)	Time of treatment*	Number of extra larval molts	Number of dead larvae	Number pupating	Number of larval-pupal intermediates	Time of ecdysis into pupae or intermediates†
0	1	0–2	0	10	0	1–7
0	5	0–2	0	10	0	1–4
0.00001	1	0–4	0	10	0	5–17
0.00001	5	0–4	0	10	0	11–19
0.00001	9	0–4	0	9	1	5–16
10	1	2–5	0	10	0	6–28
10	3	1–5	3	6	1	10–27
10	5	2–6	2	8	0	8–20
10	7	2–6	1	9	0	7–19
10	9	1–5	0	9	1	7–27
10	11	0–3	0	2	8	2–8
10	12 (prepupa)	0	0	10	0	1

+ Ten insects were used in each assay.
* Time of application in days after the final larval ecdysis.
† Time of ecdysis is given in weeks after the application.

some of them lived more than four months longer than the controls (the whole life cycle normally lasts two and a half months). The first ecdysis following application of JHa was significantly delayed. The extra larval instars appeared as normal larvae although some of them seemed to have fewer hairs. Prolongation of larval life was not accompanied by an increase in size despite the continued feeding of the larvae (the amount of consumed food was not established). Their body weight periodically fluctuated around 3.5 mg in relation to the molting cycles: it was at its lowest just after the ecdysis but increased slowly throughout the following instar, reaching a maximum shortly before the succeeding ecdysis.

Most superlarvae eventually either died or pupated. Death occurred mostly in the intermolt period and its immediate cause was unknown. Some insects died at ecdysis because they could not free themselves from the old exuvia. Superlarvae that managed to pupate usually produced morphologically perfect adults. A very few molted into pupal-adult intermediates.

Intermediates between larva and pupa were rarely observed except when the larvae were treated at the very end of the instar (11 days after the last larval ecdysis). Even in this case two individuals molted into superlarvae which eventually pupated (Table II). When JHa was administered to older animals (prepupae, 12 days after the last larval ecdysis), normal-looking pupae resulted, but these pupae developed into the pupal-adult intermediates. These intermediates resembled those produced by applying the compounds to freshly molted pupae but many of them possessed some well developed pupal hairs. Formation of a second pupa perfect in every respect, however, was also never observed.

The larval-pupal intermediates from different experiments could be arranged into a continuous series of transitions between the larval and pupal appearance. To facilitate their description, they were divided into several categories (Table III).

TABLE III

Scoring system for assessing juvenile effects in larval assays

Score	Characteristic features	
	Trogoderma granarium	*Caryedon gonagra*
0	Normal pupa.	Normal pupa.
1	Pupa with incompletely developed appendages; tarsi are larval; antennae shorter and simpler than in pupae; mouth-parts intermediate between larva and pupa.	Pupa with unusually long prothorax and larval-like tip of abdomen maintaining the larval motility.
2	Pupal-like intermediate with larval legs and atypical head bearing both larval and pupal features	Pupal-like intermediate with nearly perfect pupal wings and appendages but with a larval-like abdomen.
3	Rather pupal-like body shape, long wings, intermediate hair pattern on the dorsal body side, and nearly larval head and appendages.	Pupal-like thorax, considerably differentiated wings and appendages, and larval abdomen.
4	Larval-like intermediate with everted wings reaching $\frac{1}{3}$ of their pupal length and slightly rounded shape of the body.	Larval-like intermediate with small everted wings, slightly differentiated appendages and thoracic segments; head not retracted as much as in a normal larva.
5	Perfect superlarva but no increase in body size.	Perfect superlarva never obtained.

The larval-like intermediates shed the old exuvia while the pupal-like ones did not. The intermediates of all categories, however, usually developed further into creatures displaying a combination of larval, pupal, and adult features. The amount of imaginal differentiation seemed to depend on the type of the intermediate and, probably, on the concentration of JHa left in the body. Some intermediates, in particular those of scores 1–2, maintained their original appearance. The epidermis produced the pupal-like cuticle with hairs and the appendages kept the larval-like form. The only organs undergoing imaginal differentiation were the eyes; these remained small but formed pigmented ommatidia.

The majority of larval-pupal intermediates, particularly those that developed from the superlarvae, attained a more adult appearance during subsequent development. Some parts of the epidermis secreted adult cuticle and the number of pupal hairs was greatly reduced in other areas where a pupal type cuticle had formed.

The elytra were often sclerotized as in adults and the compound eyes attained adult shape and color. The appendages and wings remained small and undifferentiated and did not change their position. Consequently, some larval-pupal intermediates of score 1 developed into nearly normal adults except for the presence of larval appendages.

(3.) *Action of JHa on* Caryedon *larvae*

Table IV records the responses of last instar larvae of *Caryedon* to one of the most active analogues (compound No. XXIV): (1) Some larvae reduced their feeding, survived up to a fortnight after the treatment, but eventually died; (2) The larvae molted into the larval-pupal intermediates; (3) The larval life was prolonged but the insects eventually pupated; (4) The pupation was delayed only slightly or not at all, whereas the pupae developed into the pupal-adult intermediates.

Larval death or prolongation of larval life occurred when the compounds were administered at any time before the larvae began spinning. The prolongation of larval life was never accompanied by extra larval molts or any noticeable increase in the body size. The larval-pupal intermediates (Fig. 2d and 2e,) were mostly produced by applying the substances within the second half of the last larval instar, preferably to larvae spinning cocoons. On the other hand, applications to prepupae (pharate pupae) did not often affect the larval-pupal transformation but caused derangements in the following pupal-adult transformation, *i.e.*, the insects molted into pupae that developed into pupal-adult intermediates similar to those obtained by treatment of freshly ecdysed pupae (Table I). One of the prepupae developed

TABLE IV

Development of Caryedon *larvae treated with 3,4-methylendioxyphenyl 6,7-epoxygeranyl ether (compound No. XXIV)*[+]

Dose (μg)	Age of larvae*	Number of dead larvae	Number of larval-pupal intermediates	Number of pupae	Number of pupal-adult intermediates†
0.1	young	0	0	10	0
0.1	old	0	2	8	0
0.1	spinning	0	3	7	0
0.1	prepupae	0	1	9	1
1	young	6	1	2	0
1	old	0	2	8	1
1	spinning	0	4	6	1
1	prepupae	0	6	2	1
50	young	6	0	3	0
50	old	0	6	4	0
50	spinning	0	8	2	0
50	prepupae	0	6	4	3

[+] Nine to ten larvae were tested in each assay.

* The last instar larvae were divided into the following categories: young—first third of the instar (about 6 days); old–second third of the instar; spinning—period encompassing two days after the start of spinning; prepupae—insects in cocoons (0–3 days before the pupal ecdysis). The total length of the last larval instar was 17 days.

† The difference between number of pupae and that of pupal-adult intermediates indicates how many pupae molted into normal adults.

into a nearly perfect second pupa. It differed from a normal pupa by having small and pigmented adult eyes.

The larval-pupal intermediates were scored as in the case of *Trogoderma* (Table III). In contrast to the latter, only the most pupal-like intermediates (score 1) succeeded in escaping from the larval exuvia. Many intermediates developed further into creatures possessing combinations of larval, pupal, and adult features. The most larval-like intermediates ecdysed into larval-like creatures with adult cuticle restricted to the head and tiny regions on the thorax and with small but otherwise perfect adult eyes. The most pupal-like intermediates formed rather adultoid creatures possessing an unusually long prothorax, large patches of a pupal-like cuticle on the abdomen, and occasionally undifferentiated tips of appendages, particularly the antennae. The abdomen apparently maintained the larval musculature and muscle innervation because it often was as movable as in a normal larva.

(4.) *Administration of analogues as vapors*

Amounts of 0.1 to 10 mg of analogues Nos. VII and XXIV were allowed to evaporate in Petri dishes of 15 ml volume. The insects (Khapra beetle was used in these experiments) were exposed to the vapors for 6 weeks. The results are summarized in Table V.

All of the last instar larvae underwent 1 to 4 extra larval molts and eventually molted either into the larval-pupal intermediates or into normal pupae. Some pupae produced the pupal-adult intermediates, others developed into morphologically normal adults. Their fecundity was not examined.

Insects exposed to the vapors only from the start of the pupal instar mostly developed into normal adults, presumably because they passed the sensitive period

TABLE V

Effects of the vapors of JHa on the larvae and pupae of Trogoderma granarium[+]

Compound (No.)	Dose* (mg)	Number of extra larval molts	Number of larval-pupal intermediates	Number of pupal-adult intermediates	Number of adults
Exposure of larvae beginning midway the last instar					
VII	10	2–4	4	6	0
VII	1	2–3	4	5	1
VII	0.1	1–3	0	9	1
XXIV	1	1–2	0	0	10
Exposure beginning just after the pupal ecdysis					
VII	10	—	—	4	6
VII	1	—	—	2	8
VII	0.1	—	—	0	10
XXIV	1	—	—	0	10

[+] Ten insects were tested in each assay.

* The indicated amount of JHa was impregnated into filter paper and the latter placed in a covered Petri dish of 15 ml volume. Exposure of the insects began 2 hrs later.

before there could be sufficient uptake of analogue from the vapor phase. Their fertility, however, was considerably lower than in normal insects. For example, the adults which had developed in the presence of 10 mg of compound VII deposited only 4 eggs per female compared with 34 eggs in the control. The hatchability of the eggs removed from further contact with the vapors was approximately 25%, so that each female produced only one offspring. Furthermore, the hatched larvae mostly died within the first two or three larval instars.

The filter paper soaked with JHa seemed to produce effective concentrations of the vapor for a considerable length of time. Thus, insects which were placed in the dishes as late as 6 weeks after the administration of the compound were affected almost to the same degree as in the experiments just described.

Discussion

The larval-pupal transformation of both *Trogoderma* and *Caryedon* was prevented or deranged by the application of JHa during a considerable part of the last larval instar. High doses of JHa were effective even if administered to prepupae shortly before the secretion of the pupal cuticle. Late application did not impede the formation of pupae but often caused the latter to form pupal-adult intermediates. The pupal-adult intermediates were also produced by administering the compounds within the first third of the pupal instar.

Similar periods of sensitivity also have been found in other beetles. Thus the larval-pupal transformation of *Tenebrio molitor* is deranged by implanting active corpora allata into mature, last instar larvae (Radtke, 1942). So also the pupal-adult transformation is affected by administering JHa at the beginning of the pupal instar of *Tenebrio molitor* (Bowers and Thompson, 1963; Schmialek, 1963; Socha and Sehnal, 1972), *Dermestes vulpinus* (Sláma, Hejno, Jarolim and Sorm, 1970), *Tribolium confusum* (Mori, 1971), and other beetles.

A typical characteristic of both larval-pupal and pupal-adult intermediates is the predictable pattern in which the new features appear and spread over the body while the old ones simultaneously regress. The distribution of "metamorphosed" and "non-metamorphosed" tissues obviously depends on the progress of determination of metamorphosis which is known to be dependent upon determinative cell divisions (Hinton, 1963; Krishnakumaran, Berry, Oberlander and Schneiderman, 1967; Sehnal and Novák, 1969; *etc.*). The sensitivity of tissues to JHa is lost after the determinative cell divisions have been completed.

The first organs failing to respond to JHa administered to the larvae investigated in the present study were the eyes and wings and in the case of *Caryedon* also the legs. The legs of *Caryedon* and the wings and epidermis of either species lost sensitivity to JHa over a prolonged period of time. For example, certain regions of the epidermis failed to respond to an early application of the hormone while other regions were still affected by administrations to prepupae. The appendages of *Trogoderma* and the tip of the abdomen of *Caryedon* maintained their sensitivity longer than any other body part.

Comparing the larval-pupal intermediates of *Trogoderma* and *Caryedon* we find that the pattern of determination of the metamorphic changes is related to the differences between larva and pupa. For example the larval-pupal transformation of the head and appendages in *Trogoderma* seems to be relatively simple and is both

determined and accomplished within a short period of time at the end of the last larval instar (application of JHa at this time provoked development of "pupae with larval legs"). On the other hand, the larval legs of *Caryedon* seem to degenerate while the pupal legs develop from the imaginal discs. The determination of the disc development encompasses a long period of time; the successive steps of the leg differentiation appear to be determined consecutively (all various larval-pupal intermediates possessed legs with both larval and pupal features).

During the pupal-adult transformation, the organs differentiate in their fine structure and develop musculature and innervation. In both species, the loss of capacity to secrete pupal cuticle and, simultaneously, the attainment of ability to secrete adult cuticle followed a similar pattern as has been established in *Tenebrio molitor* (Rose *et al.*, 1968; Socha and Sehnal, 1972). The determination was accomplished within the first third of the pupal instar. Some changes, such as the development of adult eyes, segmentation of appendages, and in *Trogoderma* also the loss of pupal hairs, seemed to be determined prior to the pupal ecdysis. None of these changes were prevented by administering JHa after the pupal ecdysis.

The responses of larvae to JHa suggest that different insects possess diverse mechanisms for preventing lethal developmental derangements that could occur in normal development if the control of JH secretion by the corpora allata failed. One can tentatively distinguish the following types of these mechanisms: (1) The mature larvae undergo "stationary" larval molts with no increase in the body weight as long as the titer of JH remains high. When the titer decreases, the insects pupate. This is the case of *Trogoderma granarium*. (2) The mature larvae undergo extra larval molts accompanied by an increase in the body size that partly compensates for the delay in pupation. This is the case of *Tenebrio molitor* (Radtke, 1942; Schmialek, 1963) and *Galleria mellonella* (Sehnal, 1971). (3) The mature larvae do not molt as long as the JH titer remains high. In some instances they might continue to grow but only within narrow limits. This is the case of *Caryedon gonagra*.

The action of juvenile hormone has been called the "*status quo*" effect (Williams, 1961). The term is pertinent, because both JH and JHa preserve the existing stage of development. The larval-pupal intermediates, which are produced by impeding further progress of metamorphosis in the middle of the larval-pupal transformation, maintain the capacity to molt and are thus capable of further development. In the absence of further JHa they should theoretically produce pupal-adult intermediates; the original larval tissues would metamorphose into pupal ones and the original pupal tissues into the adult ones. If the titer of JHa remains high, the larval-pupal intermediates should maintain the *status quo* and appear after ecdysis the same as before.

The intermediates without further supply of JHa indeed developed towards the adult stage. Some body parts, however, always metamorphosed more than the others. This suggests that the sensitivity of different tissues to JHa diversified; differentiation of some tissues was hindered by the remnants of JHa in the body whereas other tissues differentiated despite the presence of JHa.

The results of our tests confirm that certain changes in the chemical structure of analogues significantly alter the biological activity. The comparison of these data with the information on *Tenebrio molitor* (reviews Williams, 1970; Sláma, 1971), *Dermestes vulpinus* (Sláma *et al.*, 1970) and *Tribolium confusum* (Bowers,

1971; Mori, 1971) indicates the relationships between the chemical structure and the activity on different coleopteran families. For example, the activity of the farnesane-type compounds seems to depend on the following parts of the molecule:

(1) The number of double bonds—the beetles respond most readily to substances having 2, 3 and 6, 7 double bonds (Wakabayashi, Sonnet and Law., 1969; Sláma *et al.*, 1970). The present results demonstrate that the shift of the 6, 7 double bond to the 4, 5 position does not considerably alter the activity.

(2) Hydrochlorination or epoxydation of the 10, 11 double bond generally increases the activity (Bowers, Thompson and Uebel, 1965; *et al.*, 1970; Mori 1971). *Trogoderma* seems to be particularly sensitive to the 11-chlorine derivative. Compounds with the methoxy group on C-11 appear to be less active.

(3) Prolongation of the side chain on C-11 also increases the activity (Röller and Dahm, 1968; Wakabayashi *et al.*, 1969). Substances with two ethyl groups on C-11 were reported to be even more active on *Tenebrio* and *Tribolium* (Mori, 1971).

(4) Additional substitution on C-7 generally decreases the activity (Sláma, Romaňuk and Sorm 1969). The diethylamid of 6, 10-dihydro-7, 11-dichlorofarnesoate (not tested in the present study), however, proved to be very active on *Tenebrio* (Cruickshank, 1971).

Similar relations between chemical structure and biological activity may be found in the group of aromatic JHa. The most active of all compounds tested on *Trogoderma* and *Caryedon* appears to be, 3,4-methylendioxphenyl 6,7-epoxygeranyl ether. The compounds of this type are also very active on *Tenebrio* (Bowers, 1969) and *Tribolium* (Bowers, 1971). Some aromatic substances may act rather specifically only on some families of Coleoptera. For example, 4-nitrophenyl 7-ethoxygeranyl ether (XXI) is very active on *Tenebrio* (Sláma, 1971) but nearly inactive on *Trogoderma* and *Caryedon*.

Beetles of different families differ one from another by their responsiveness to certain types of analogues as well as by their sensitivity to JHa in general. For example, *Tenebrio* is generally more sensitive than *Caryedon* but, in regard to certain compounds, it is less sensitive than *Trogoderma*. Consequently, results of assays on one species cannot be extended to all Coleoptera.

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Summary

1. The metamorphosis of *Trogoderma granarium* and *Caryedon gonagra* may be deranged with as little as 0.000005 μg and, respectively, 0.05 μg of certain juvenile hormone analogues.

2. Under the prolonged influence of analogues the larvae of *Trogoderma* undergo several "stationary" extra larval molts with no increase in the body size.

The larvae of *Caryedon* do not molt but their pupation is considerably delayed. In extreme cases the larvae die.

3. Exposure of *Trogoderma* and *Caryedon* to the analogues during the last third of the last larval instar often induces development of larval-pupal intermediates. The species differ in the distribution of larval and pupal features in these intermediates.

4. The larval-pupal intermediates develop into creatures composed of larval, pupal, and adult tissues.

5. Treating the insects shortly before or after the pupal ecdysis results in formation of pupal-adult intermediates with a similar distribution of pupal and adult tissues in both species.

6. Pupal assays revealed that the investigated species differ from one another as well as from other beetles in their responsiveness to certain analogues and also in their responsiveness to the analogues in general.

7. *Trogoderma* is affected by the vapors of analogues.

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